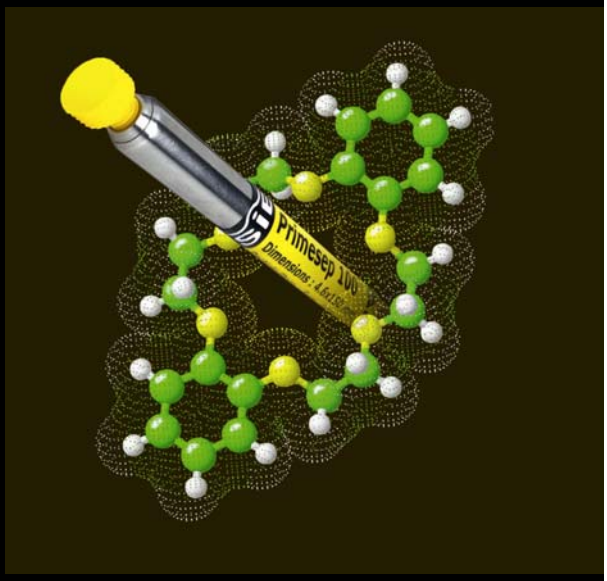
SIELC

Primesep™

Separation of Ions



"New Alternative to Ion Chromatography"

Content

Introduction

Ion-chromatography (IC) is an established technique for analysis of inorganic and some organic ions. However, there are some problems associated with this technology.

IC typically requires specific and expensive equipment which is not readily available in every analytical lab.

IC is a very sensitive technology, and in the analysis where the concentration of analyzed ions is significant, it requires several dilution steps to bring the sample to a convenient concentration.

IC perfectly works in pure aqueous media; however, the IC instrumentation can not be used with a significant amount of organic component in the mobile phase. This causes difficulties when an organic sample with a small amount of analyte is introduced in the IC column. Usually, it leads to contamination and destruction of the column, or requires some additional clean up steps.

Non-charged analytes are usually not detectable or not separable within IC conditions.

Different sets of conditions are required for separation of anions and cations, and they can not be analyzed simultaneously.

SIELC Technologies offers an alternative approach that can address the problems above.

The combination of Primesep™ mixed-mode stationary phase with the evaporative light scattering detecting (ELSD) technique allows to use a broad range of mobile phases including the combination of water with acetonitrile, or methanol with acidic modifiers such as TFA, acetic and formic acid, ammonium acetate, ammonium formate, triethylamine acetate. Using this wide variety of mobile phases, we can achieve various separations. Also, a great number of compounds can be separated using the standard HPLC equipment with the addition of ELSD only. Charged and neutral organic and inorganic analytes can be simultaneously analyzed within a single HPLC run. Direct injection of the sample without any cleanup or/and dilution is achievable.

Novel Stationary Phase Properties

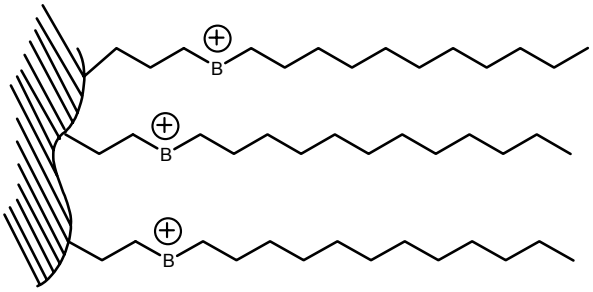
In ion-pairing chromatography, the retention of ionizable species is controlled by the concentration and type of ion-pairing reagents.

Pentanesulfonic acid, heptanesulfonic acid, sodium dodecylsulfate, tetrabutylammonium hydroxide are ion-pairing reagents that are typically used for retention of polar compounds in reverse phase chromatography. By analogy, Primesep™ HPLC mixed-mode columns are offered in several modifications of the stationary phase with different strengths of ion-bearing groups for cation exchange mode (Primesep A, Primesep 100, Primesep 200) and for anion-exchange mode (Primesep B).

In addition, a column with unique properties that allows to switch retention of neutral and ionizable compounds by changing pH of the mobile phase is offered (Primesep SWITCH™).

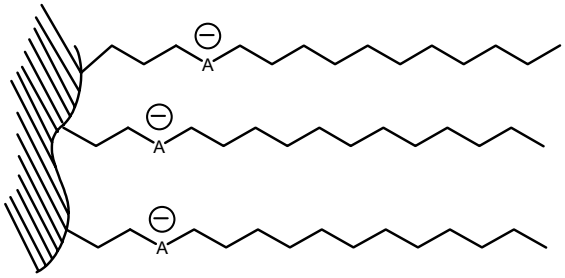
Primesep C column forms a strong complex with amines. The strength of the complex increases from tertiary to secondary, and primary amines. A pKa value for amines usually decreases in the same order. Contrary to ion-exchange separation, a reverse elution order is observed on Primesep C columns for substituted amines

Mixed-Mode Primesep™ Columns

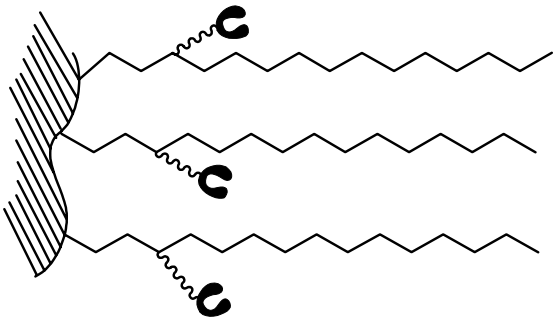


Primesep B
Primesep B2

Having an embedded ion-pairing group, the Primesep column requires no ion-pairing reagent in the mobile phase to retain and separate ionizable polar compounds.



Primesep A
Primesep 100
Primesep 200
Primesep 300



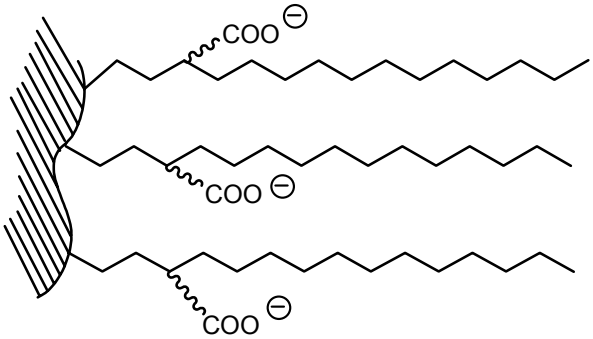
A newly developed Primesep C column (C stands for "complex") forms a weak complex with amino compounds and metal ions. With a reverse stationary phase as a basis for primary interaction, the column offers a typical RP retention profile for neutral compounds. In addition, embedded hosting groups interact with amines and other ions, and form a unique retention pattern. Amines with equal hydrophobicity retain on Primesep C in the following order: tertiary<secondary<primary. Alkali metals are retained in the order: K+<Na+<Li+ , which is a reverse order compared to the classical ion-exchange.

SWITCH™ Phase Technology

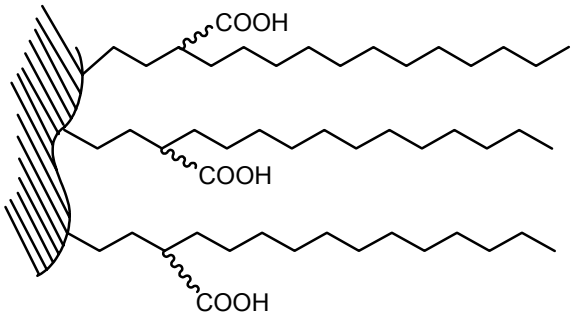
Columns based on SWITCH Phase™ technology change their properties depending on pH of the mobile phase. Embedded carboxylic acid is fully ionized at pH above transition point and loses charge when mobile phase pH goes below transition point. By controlling pH of the mobile phase, the polar properties of the stationary phase can be altered to tune your separation needs.

Primesep 300	Transition @ pH=3
Primesep 200	Transition @ pH=2
Primesep 100	Transition @ pH=1
Primesep A	Transition @ pH=0

Primesep 300 at pH > 3.5



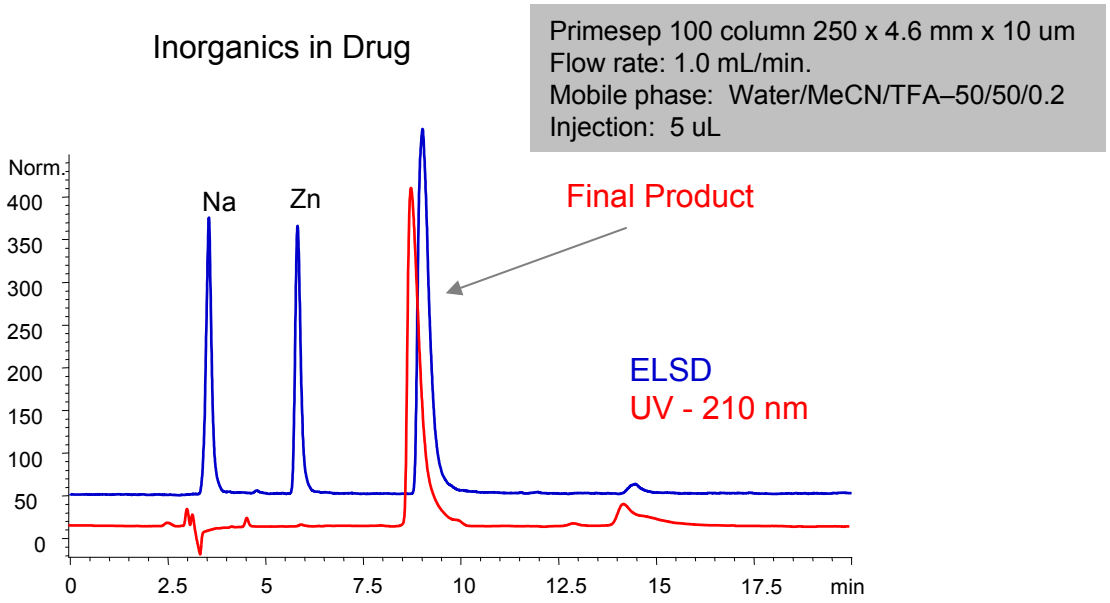
Primesep 300 at pH < 2.5



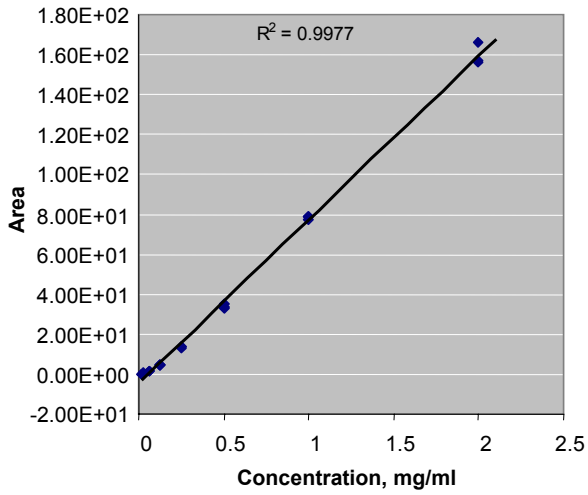
Simultaneous Separation of Inorganic Ions and Organic Compounds in Single HPLC Method

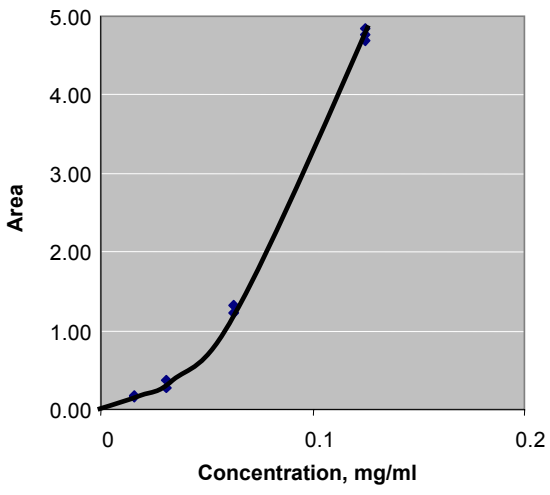
In many instances, ionizable compounds exist as salts of organic molecules with inorganic counter ions. This is common for drugs, surface active compounds, biological molecules, and many other industrial and research substances. Typically, two independent analytical methods are created for analysis of these salts – reverse phase for organic parts and ion chromatography method, or titration, for inorganic parts.

Primesep™ columns offer unique ability to analyze both parts of such salts at the same time. ELSD in combination with standard UV detector is a convenient tool for the purpose.

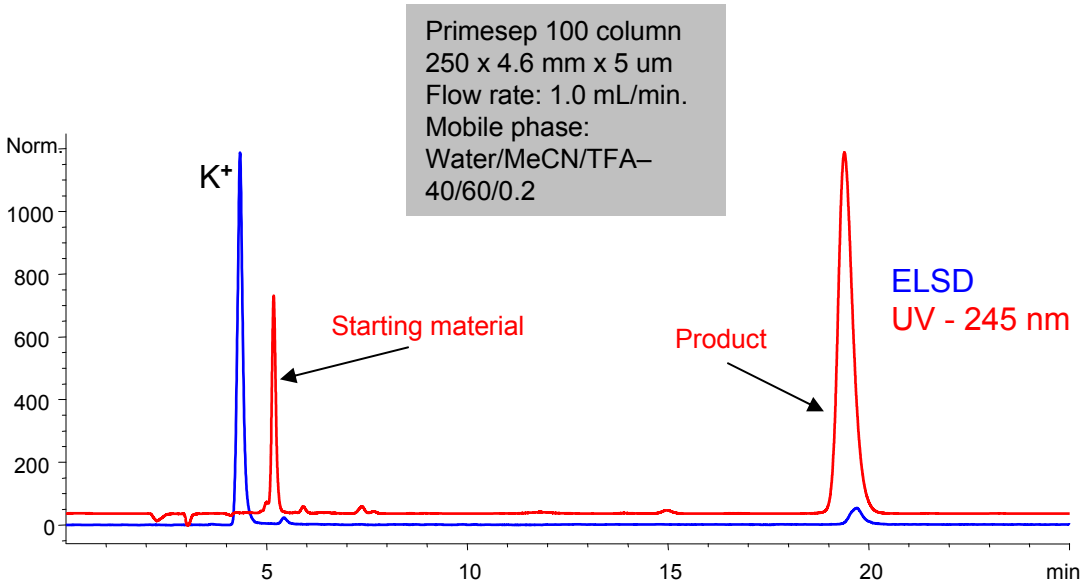


Quantitation of inorganic components of the mixture can be performed using an ELSD signal. This detector is inherently not linear, but if the concentration of inorganic ion is significant, which is usually the case in pharmaceuticals, then a significant linear region can be exploited with the ELSD technology. At low concentration a non-linear treatment of calibration curve should be used to get accurate results.

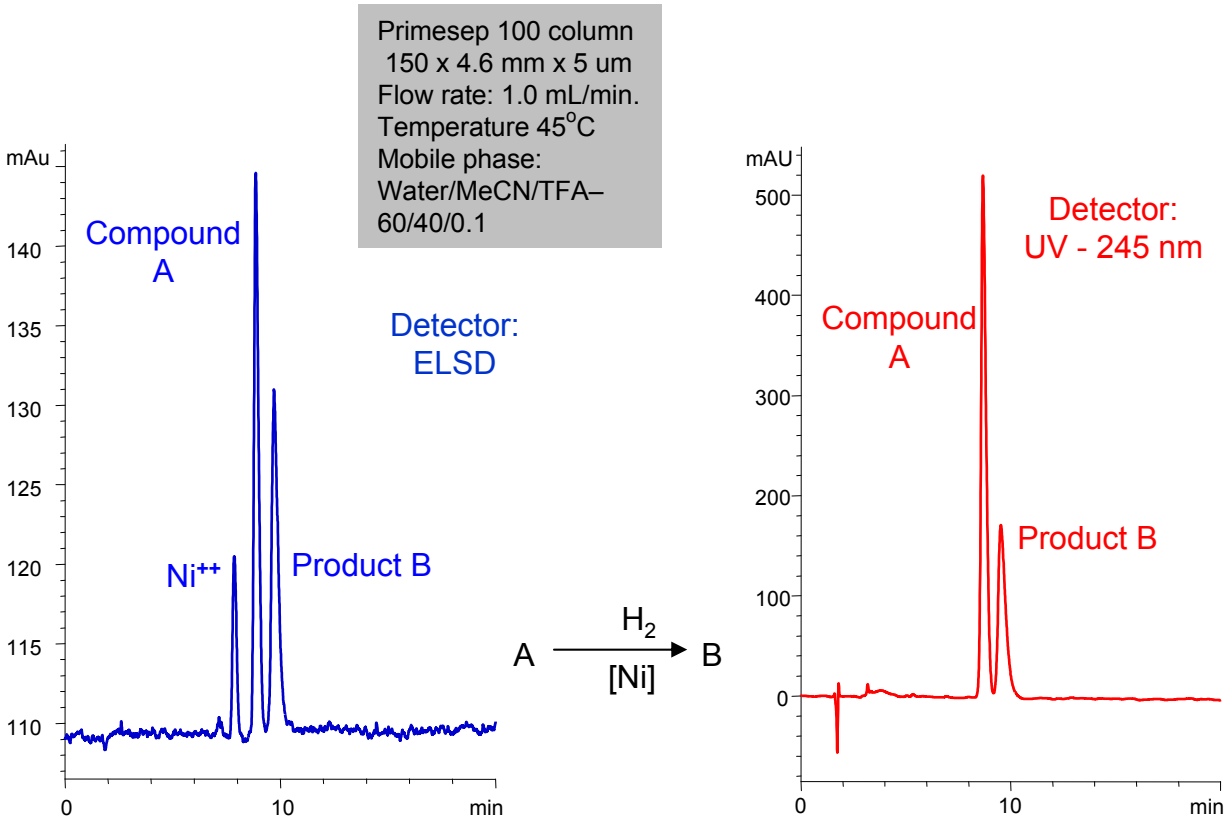




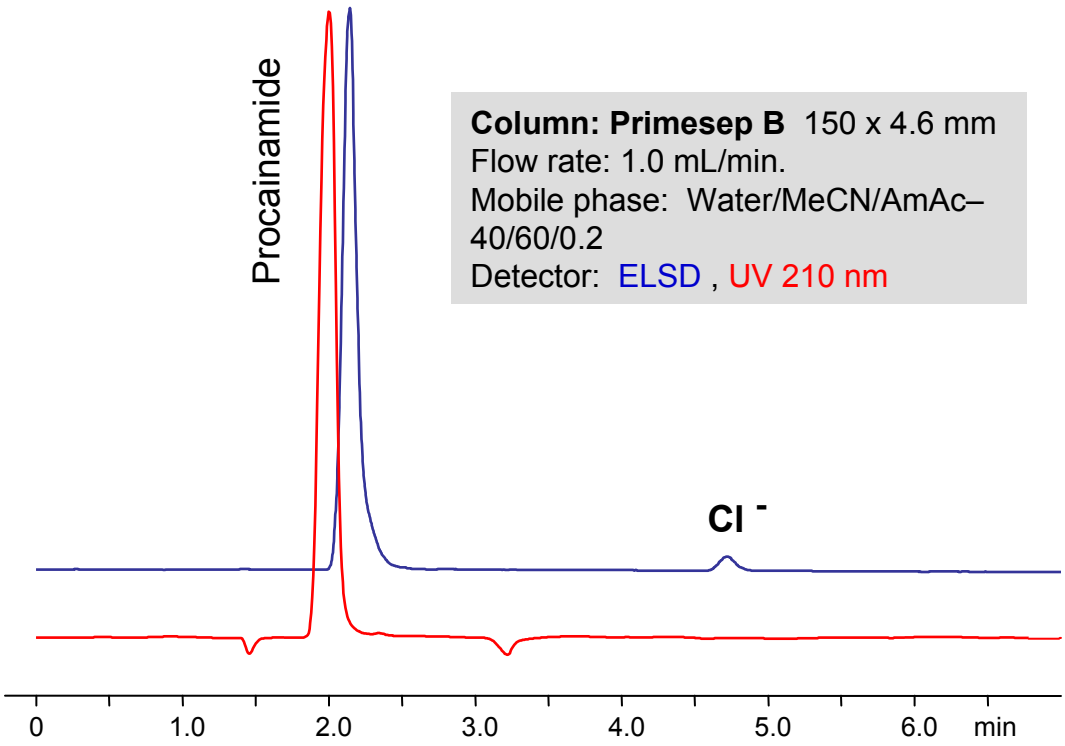
Quantitation of Potassium



Analysis of Nickel in Hydrogenation Reaction

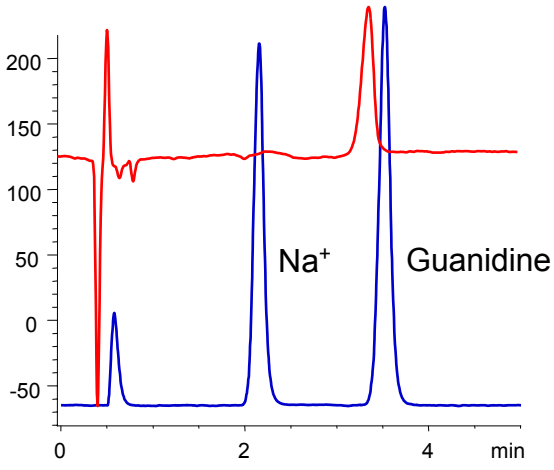


Analysis of HCl Salt of Pharmaceutical Compound



Column: Primesep B 150 x 4.6 mm
Flow rate: 1.0 mL/min.
Mobile phase: Water/MeCN/AmAc– 40/60/0.2
Detector: ELSD , UV 210 nm

Guanidine Analysis



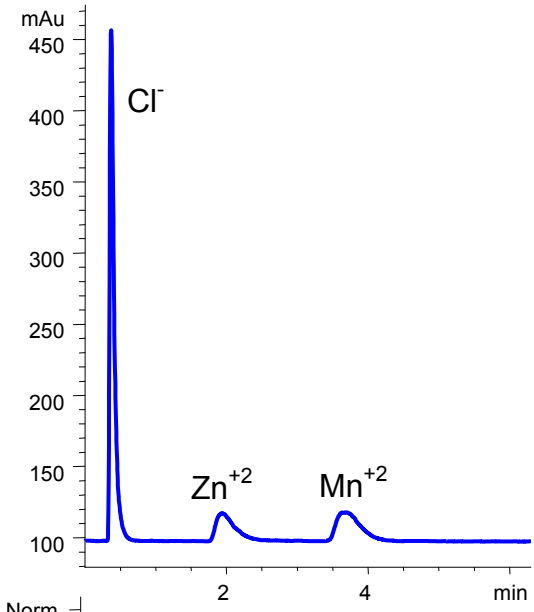
Column: Primesep 100 50 x 4.6 mm 5 um
Mobile phase: MeCN/H_2O/TFA – 25/75/0.05
Flow rate: 1.0 ml/min
Detector: ELSD , UV 200 nm

Retention of Polar Compounds without Ion-Pairing Reagents

While being a technique of choice to solve separation problems in many industries, HPLC, and the reverse phase mode in particular, has its limitations. One of the weak points of reverse phase is the lack of retention of highly polar compounds on conventional stationary phases. Traditionally, mobile phase additives, such as ion-pairing reagents, have to be employed for separation of these compounds. Practical limitations of ion-pairing chromatography include occurrence of artifact when using gradient elution, incompatibility with MS, ELSD, preparative chromatography, and complex mobile phase preparations.

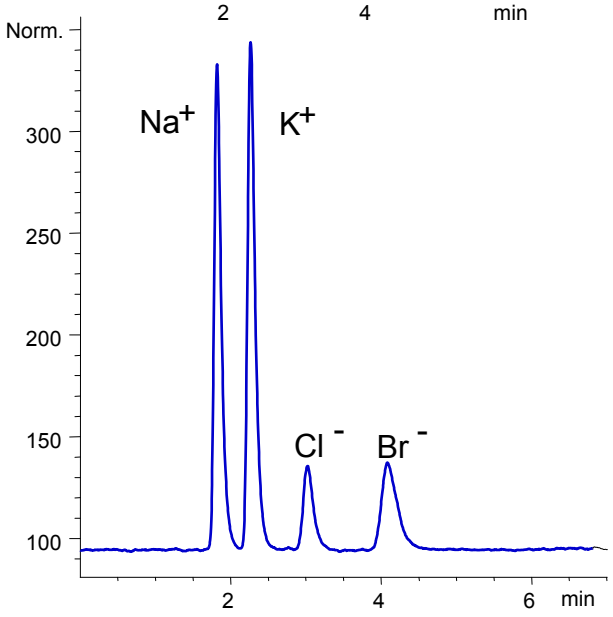
Primesep™ mixed-mode stationary phases are suitable for separations of polar and non-polar compounds at both analytical and preparative scales in isocratic and gradient modes. These stationary phases allow for a great degree of flexibility in the separation of a broad range of analytes on one stationary phase platform utilizing simple mobile phases that are compatible with multiple detection modes.

Separation of Inorganic Ions



Ammonium acetate based buffer offers an ability to see and quantitate anion like chloride in ELS detection mode. Additionally, it modifies the column surface and changes the selectivity. Zn and Mn ions are co-eluted with TFA based mobile phase.

Column: Primesep 100
50 x 4.6 mm x 5um
Flow rate: 1.0 mL/min.
Detection: ELSD
Mobile Phase: 70% MeCN + 30% NH_4Ac 30 mMol pH 4.0



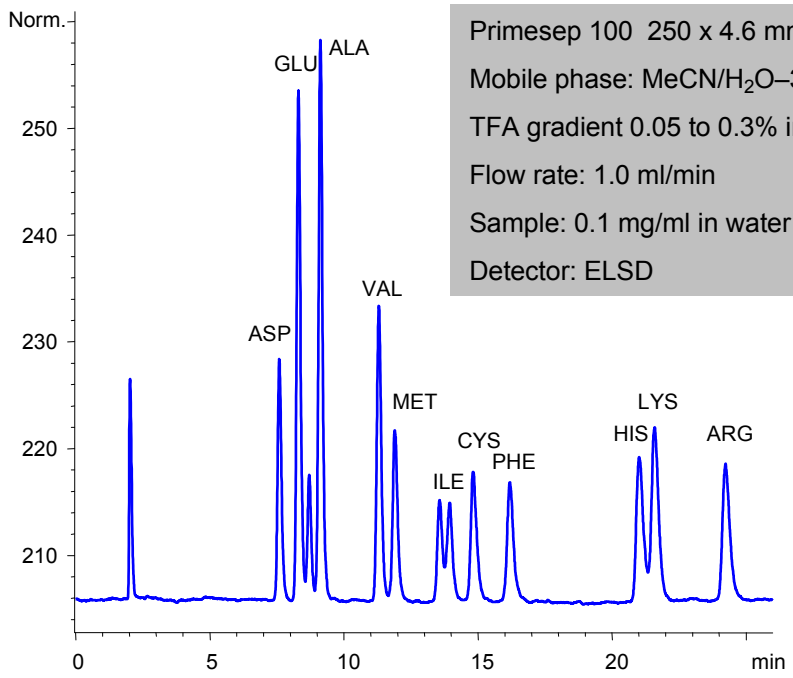
Positively and negatively charged inorganic ions can be simultaneously separated and quantitated by using dual column system and ELSD

Column: Serial connected Primesep 100, 50 x 4.6 mm x 5μm with Primesep B, 50 x 4.6 mmx 5 um
Flow rate: 1.0 mL/min
Mobile Phase: H_2O/MeCN – 70/30 with NH_4Ac 50 mM pH 5.0
Detector: ELSD
Injection: 5 uL
Sample: KBr 0.5 mg/ml; NaCl 1.0 mg/ml in water

Analysis of Underivatized Amino Acids

The presence of the ion-exchange groups on a Primesep™ column makes it a perfect choice for separation of underivatized amino acids.

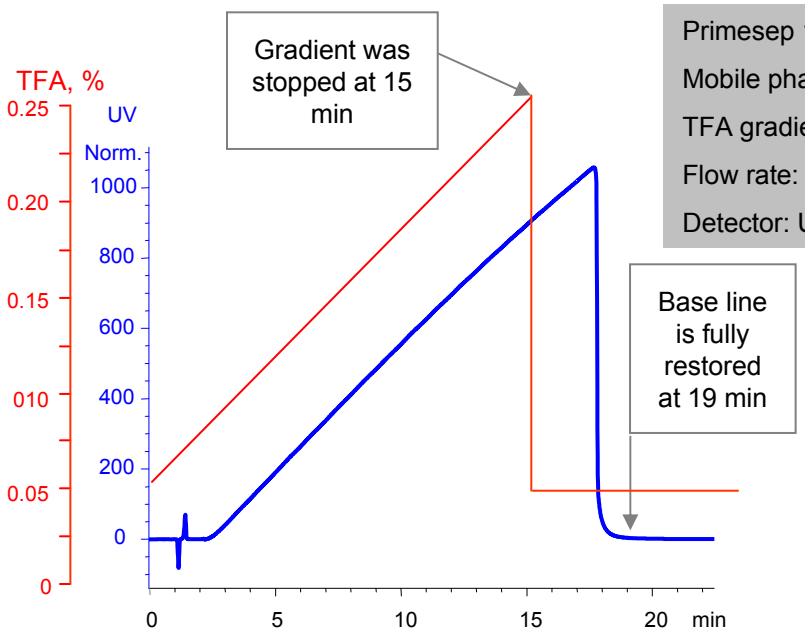
Acid gradient allows separation of compounds with significantly different pKa within a single chromatography run.



Primesep 100 250 x 4.6 mm
Mobile phase: MeCN/H_2O–30/70
TFA gradient 0.05 to 0.3% in 25 min
Flow rate: 1.0 ml/min
Sample: 0.1 mg/ml in water
Detector: ELSD

Extremely Fast Equilibration Time with Acid Gradient

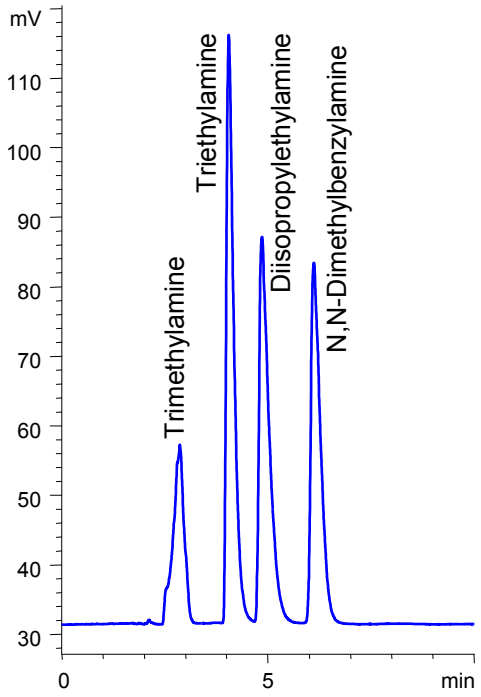
The presence of the acidic groups on a Primesep™ column prevents retention of an acid on the stationary phase. It results in quick equilibration time equal to 2-3 column volumes. Thus, an acid gradient is a convenient option for separation of compounds with a drastically different pKa value.



Primesep 100 150 x 4.6 mm
Mobile phase: MeCN/H_2O–15/85
TFA gradient 0.05 to 0.25% in 15 min
Flow rate: 1.0 ml/min
Detector: UV 210 nm

Ion-Exchange and Hydrophobic Mechanism in Tertiary Amines Separation

Strong basses such as tertiary amines retain too strongly on Primesep A or Primesep 100 columns. Primesep 200 is a weaker cation exchanger than Primesep 100 and Primesep A, and it separates strong bases in mild conditions.



Primesep 200 column 150 x 4.6 mm x 5 um

Mobile phase: MeCN/H_2O/TFA–20/80/0.15

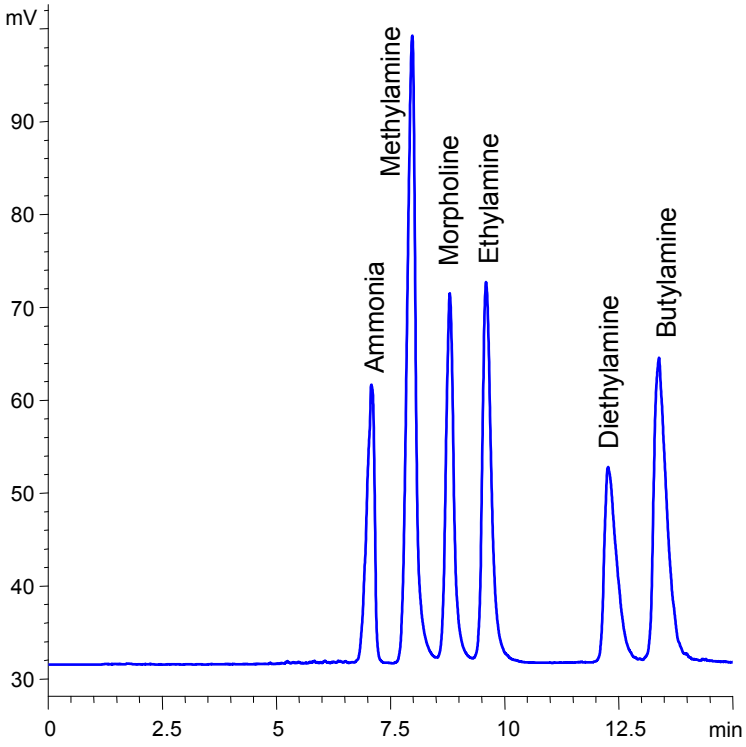
Flow rate: 1.0 ml/min

Injection: 5 ul

Sample: 3.0 mg/ml each

Detector: ELSD, (Temperature 35°C)

Ion-Exchange and Hydrophobic Mechanism in Amines Separation



Primesep A column 150 x 4.6 mm x 5 um

Detection: ELSD, (Temperature 35°C)

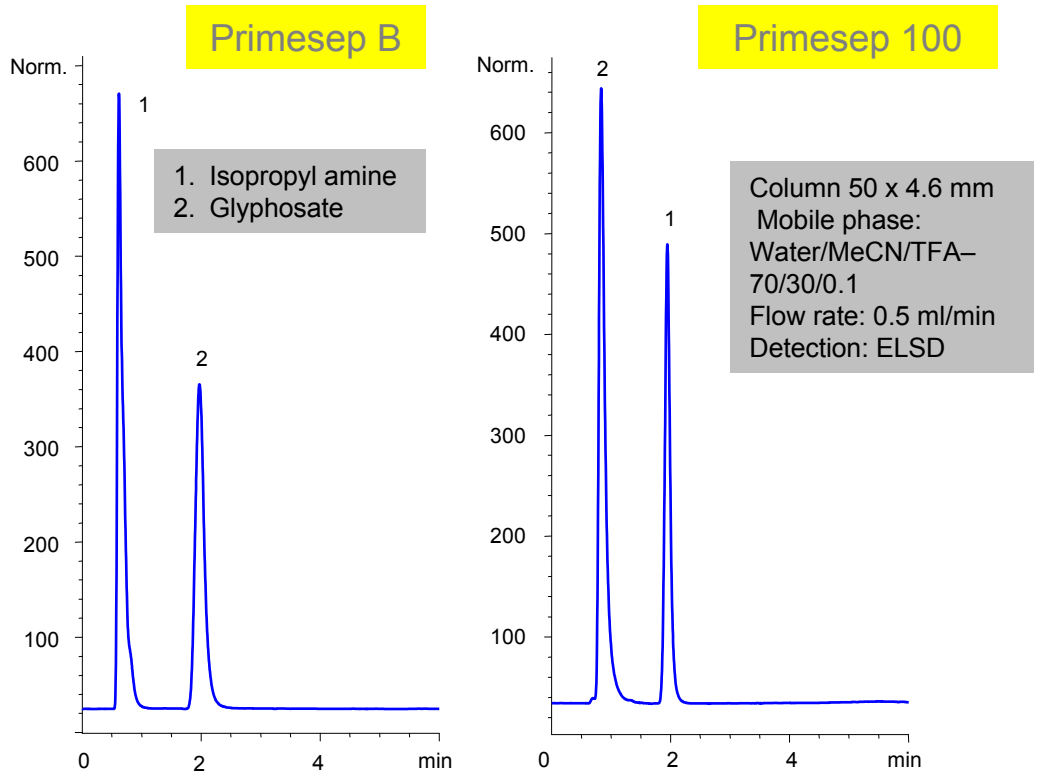
Mobile phase: MeCN/H_2O–15/85

TFA gradient 0.05 to 0.25% in 15 min

Flow rate: 1.0 ml/min

Sample: 1.0 mg/ml each

Ion-Exchange Mechanism in High Organic Mobile Phase. Glyphosate Analysis.



Separation of Quaternary Amines

Quaternary amines are strong basses. They are not volatile and can not be analyzed by GC. A typical HPLC separation will result in no or very little retention for these polar molecules. Primesep C with volatile mobile phase allows to separate and quantitate quaternary amines with ELSD or MS detection technique.

Primesep C 50 x 4.6 mm x 5 um

Mobile phase: MeCN/H_2O–15/85

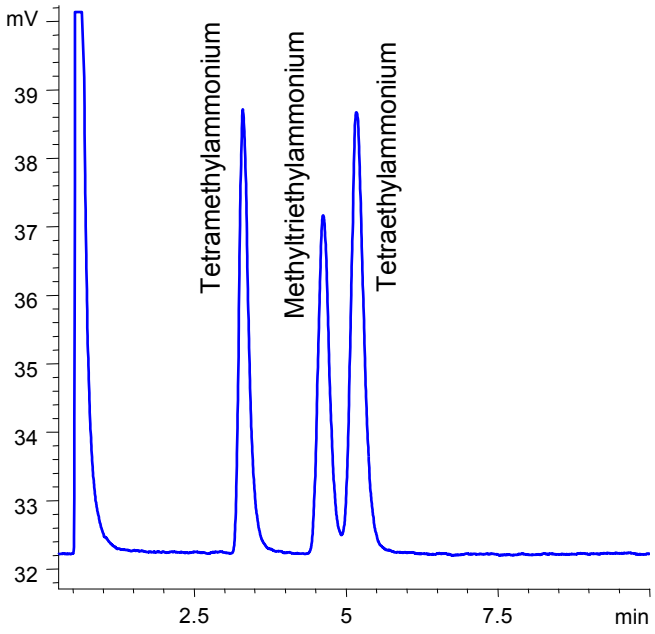
TEA acetate 20 mM pH 5.0

Flow rate: 1.0 ml/min

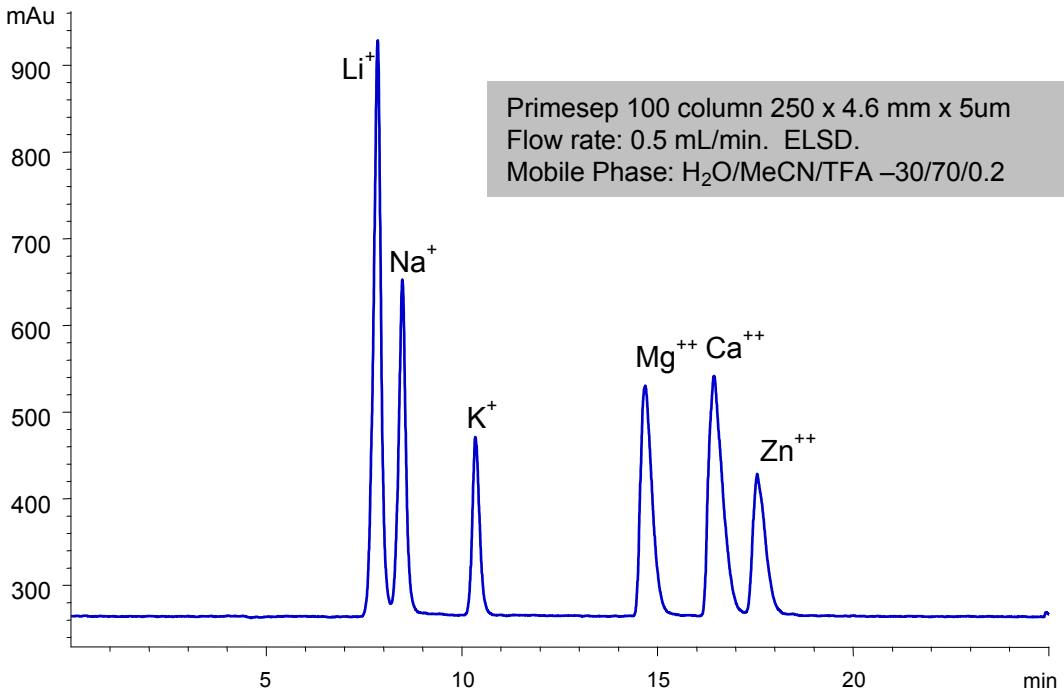
Sample: 0.6 mg/ml each

Injection: 5 mc

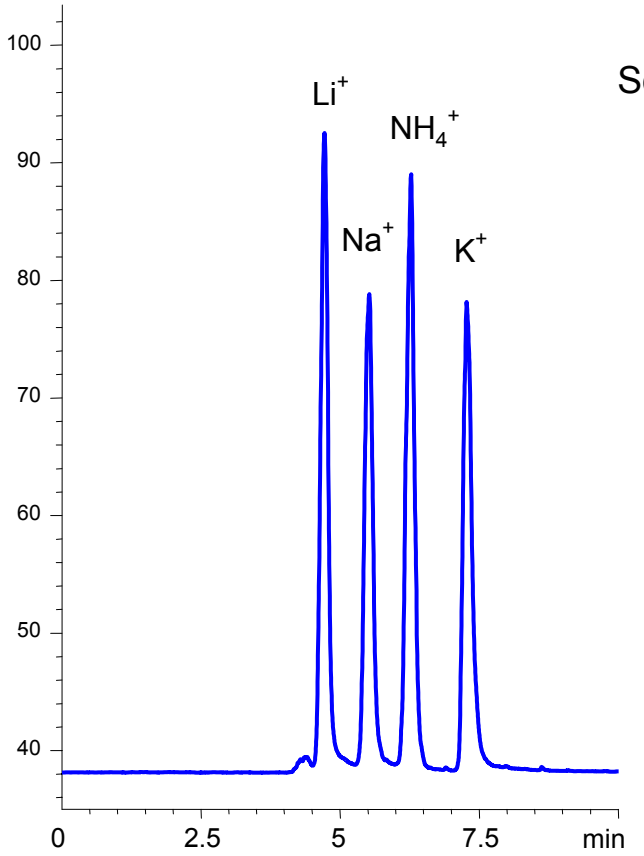
Detector: ELSD, (Temperature 35°C)



Ion-Exchange Mode. Separation of Inorganic Cations



Primesep 100 column 250 x 4.6 mm x 5um
Flow rate: 0.5 mL/min. ELSD.
Mobile Phase: H_2O/MeCN/TFA –30/70/0.2



Separation of Inorganic Cations

Column: Primesep 100
250 x 4.6 mm x 5um
Flow rate: 1.0 mL/min.
Detection: ELSD
Mobile Phase:
H_2O/MeCN/TFA –20/80/0.2

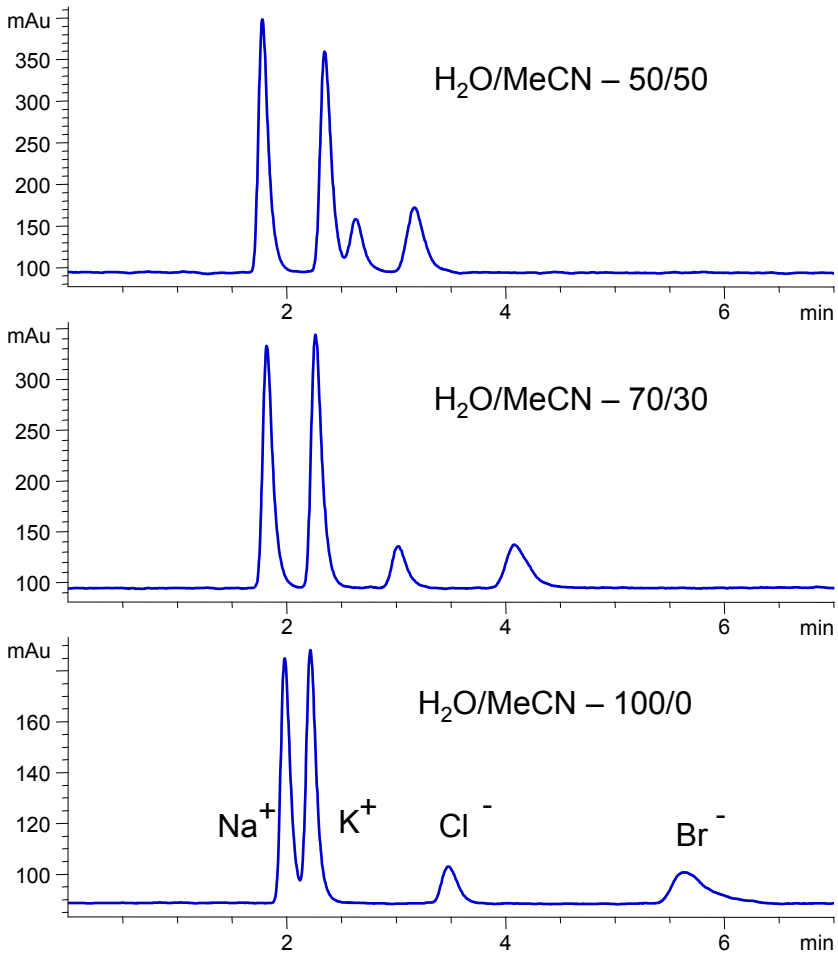
Unique Adjustable Selectivity

Primesep™ mixed-mode stationary phases provide multiple types of interactions with analytes. Ionizable compounds interact with the stationary phase by reverse-phase, ion-exchange or ion-exclusion mechanisms.

The amount of the acid in the mobile phase influences the retention attributed to the ion-exchange interaction to the same degree as the organic modifier affects the retention in reverse-phase separation. Thus, the amounts of organic and acidic modifiers are both important for control of retention of ionizable analytes. In addition to hydrophobic interactions, the neutral compounds participate in different polar interactions with highly polar column's functional group. These polar groups can be modified by selection of the mobile phase. The basic functional group on Primesep B column forms a salt with different acids residue (sulfate, perchlorate, trifluoroacetate, etc.), and each salt participates differently in polar interaction with neutral analytes.

Analytes themselves can be ionized in many ways depending on the pH of the mobile phase, and retention time of your compounds can also be substantially altered by changing the pH of the mobile phase.

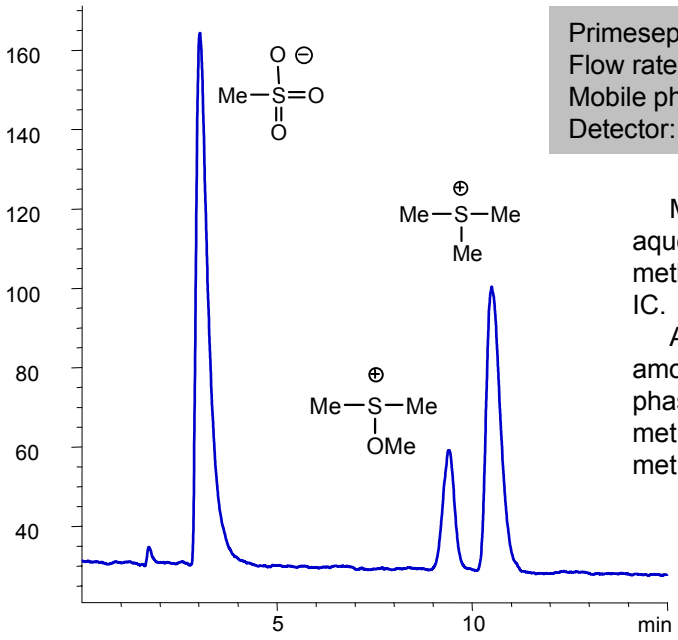
Separation of Inorganic Ions



Column: Serial connected Primesep 100, 50 x 4.6 mm x 5um & Primesep B, 50 x 4.6 mmx 5um
Mobile Phase: H_2O/MeCN with NH_4Ac 50 mM pH 5.0
Flow rate: 1.0 mL/min
Detector: ELSD
Injection: 5 uL
Sample: KBr 0.5 mg/ml; NaCl 1.0 mg/ml in water

Resolution of positive and negative ions is affected differently by the amount of organic modifier in the mobile phase.

Separation of Sulfonium Ions

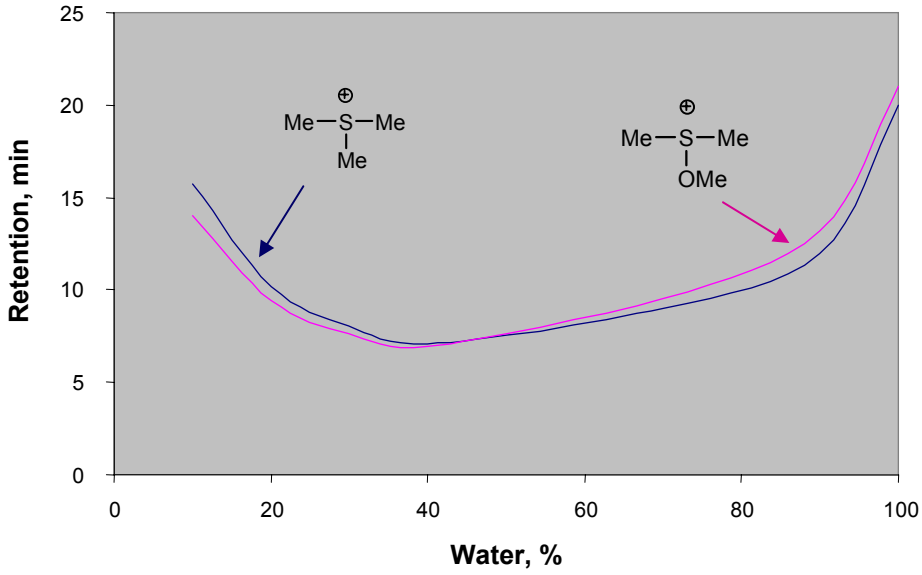


Primesep 100 column 150 x 4.6 mm x 5 um
Flow rate: 1.5 mL/min.
Mobile phase: Water/MeCN/TFA–10/90/0.1
Detector: ELSD.

Methoxysulfonium ion is not stable in aqueous solutions. There are no reliable methods to analyze this mixture by means of IC.

As Primesep™ columns work with any amount of organic modifier in the mobile phase, a condition with low water and high methanol concentration is chosen for this method.

Effect of Water Concentration

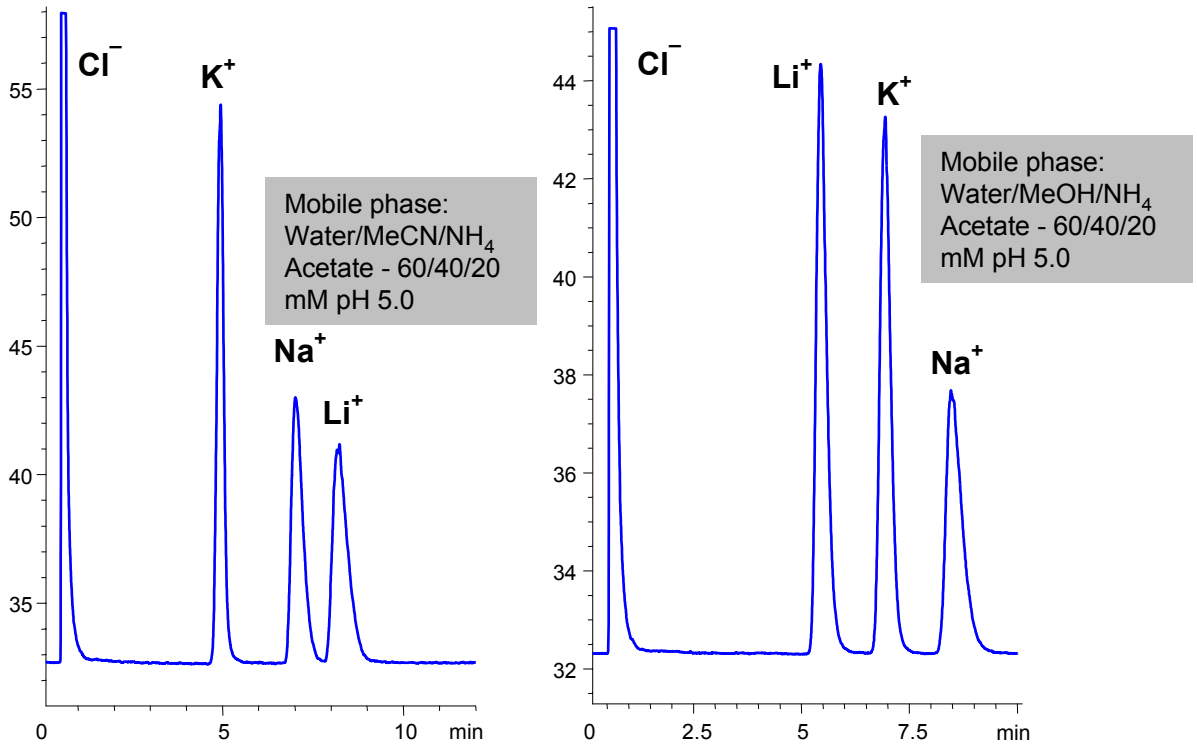


Primesep 100 column 150 x 4.6 mm x 5 um
Flow rate 1.0 mL/min.
Mobile phase: Water/MeCN with TFA 0.1%
Detector: ELSD.

New Concept in Column Chemistry – Primesep C™.

Typical ion-exchange columns would separate alkali ions in the following order: Li, Na, K. Primesep C (stands for Complex) offers unique selectivity. The ions elute on the Primesep C column in reverse order compared to ion-exchange elution. The pH working range for these columns is from 1 to 7, but their complex formation properties are substantially suppressed at the pH below 3. In order to facilitate the complex formation, the pH of the mobile phase should remain in the range of 3-7. The degree of complex formation can be adjusted by selecting the pH of the mobile phase.

Primesep C 50 x 4.6 mm Flow rate 1.0 mL/min. Detector: ELSD

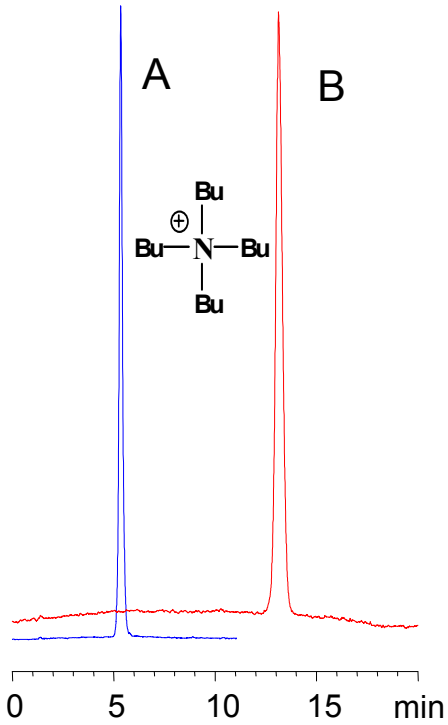


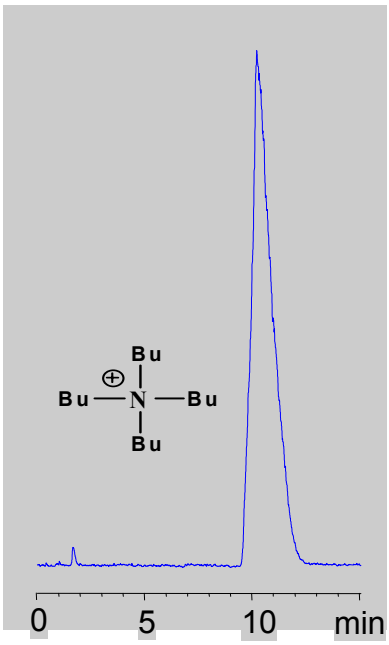
Retention of Hydrophobic Quaternary Amines

Quaternary amines with hydrophobic properties can be retained on a Primesep B column. Using the stationary phase with the same surface charge as the one of the analyte results in the superior peak shape. Even the best surface deactivated columns can not provide similar efficiency and peak symmetry.

Primesep B, 150 x 4.6 mm
Mobile Phase A:
H_2O/MeCN/TFA - 70/30/0.15
Mobile Phase B:
H_2O/MeCN/TFA - 80/20/0.15
Flow rate: 1.0 mL/min
Detector: ELSD
Peak plate count 5200
Peak symmetry 0.70

Leading brand C18 column
150 x 4.6 mm x 3.5 um
Mobile Phase:
H_2O/MeCN/TFA - 70/30/0.1
Flow rate: 1.0 mL/min
Detector: ELSD
Peak plate count 640
Peak symmetry 0.37





Conclusions

- Primesep™ mixed mode columns are a powerful separation tool for analysis of polar compounds and complex mixtures with compounds of different polarity
- Combination of Primesep™ columns with ELSD offers simple alternative to ion-chromatography
- Anions and cations can be analyzed at the same time with the same mobile phase
- Ion-chromatography with concentration of organic modifier from 0 to 100% can be performed with possibilities to adjust resolution and peaks elution order
- Inorganic and organic compounds can be analyzed at the same time using ELSD alone or in combination with UV detector